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WITH BRUCELLA ABORTUS STRAIN 19 VACCINE

(Reaktsiya i immunitet u severnykh olenei,
privitykh vaktsinoi iz shtamma 19)

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REACTION AND IMMUNITY IN REINDEER VACCINATED
WITH BRUCELLA ABORTUS STRAIN 19 VACCINE
(Reaktsiya i immunitet u severnykh olenei, privitykh
vaksinoi iz shtamma 19)

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The results of experiments on the reaction of healthy and brucellosis-stricken deer to vaccination with *B. r. abortus* strain 19 are given in this article. The intensity and duration of immunity as dose-dependent are reviewed as well.

B. r. abortus strain 19 live vaccine was injected subcutaneously in the neck region of healthy deer (150 head) of different ages and 1½-month-old calves from a brucellosis-free herd. Diseased animals were also employed. Three groups of 50 healthy deer each were injected with 6×10^{10} , 15×10^9 and 6×10^9 *B. r. abortus* respectively.

Three days after injections in the first group (6×10^{10} microorganisms), edema developed at the site of injection in 47 deer. This was 10-12 cm in diameter, febrile and resilient to touch. The animals were in depressed condition, showed no appetite and did not ruminate. Some of the deer lagged behind the herd and often lay on the ground for prolonged periods. The prescapular and intermaxillary lymph nodes were enlarged; sickly appearance and restricted movement were observed. The body temperatures rose to 40-41° for four or five days. From the fifth to tenth day the general condition improved and the edema disappeared, but a considerable portion of the deer (15%) developed abscesses at the site of injection.

The reaction of deer given 6×10^9 and 15×10^9 microorganisms was somewhat more weakly expressed. But these animals were also depressed and the body temperatures rose to 40-40.3° with edema of slightly smaller dimensions which disappeared quickly. Abscesses appeared in five deer injected with 15×10^9 microorganisms.

Of the ten calves vaccinated with 6×10^9 microorganisms, three developed abscesses with body temperatures rising to 40.1°; all the calves showed depression and poor appetite.

Deer (106 head) from the brucellosis-infected herds, with positive agglutination reaction but no clinical symptoms, had vaccine reaction no different from that of healthy animals. No variation of reaction was observed in animals of different sexes or ages.

In order to follow the development and disappearance of agglutinins in the serum of the experimental deer, serum agglutinin titers were carried out, as well as the Huddleson [opsonocytophagic] test for *B. r. abortus*,

at the onset and 3, 5, 10, 20, 30, 60 and 90 days, eight and ten months after injection. Three days after vaccination some of the deer had positive agglutination reaction in titers 1:5 and 1:10. Within ten days all deer, irrespective of sex, age or dose, had agglutination reaction titers of 1:50-1:400, and also positive Huddleson test reactions.

The agglutinin content of serum gradually increased, and after 30 days reached the maximum (1:400-1:12,800). Then the titer began to decrease in some deer, but even after eight and ten months a considerable number of deer had agglutinin of 1:50 to 1:400 (in isolated cases, 1:800 to 1:1600). After 20 to 24 months, practically all the deer had negative agglutination reactions. The rate of decrease of agglutinins is more markedly expressed in deer injected with small doses of vaccine.

Br. abortus strain 19 vaccine (60×10^{10}) was injected into deer (45 head) from the brucellosis-infected herds with negative agglutination reaction. The blood was tested 10, 20 and 30 days after vaccination. Within ten days all deer had positive agglutination reaction in titers 1:40-1:1600 (in isolated cases, 1:3200). After 30 days the serum agglutinin titer rose to 1:3200 in most of the deer.

The control deer (16 head) with positive agglutination reaction were not vaccinated. Blood examination (simultaneously with that of the vaccinated deer) demonstrated no significant fluctuation in the serum agglutinin titer. In none of the control deer was there any rise in agglutinin content.

Organic changes occurring in animals after the Br. abortus strain 19 vaccination were studied in the three groups of healthy deer. The first group was administered the full vaccine dose, the second group one-quarter and the third group one-tenth of the dose given to cows. After vaccination, at 5, 15, 30, 60 and 90 days, and eight months, two deer of each group were killed and examined.

Deer examined five days after vaccination had swollen local lymph nodes, thickened skin at the site of the injection, and a jellylike infusion in the subcutaneous tissue. After 15 days all lymph nodes were enlarged; after 30 days no macroscopic changes were observed in the viscera.

Five days after vaccination Br. abortus organisms were detected localized in local lymph nodes and in two cases in blood of the heart. After 15 days Brucella were found in the viscera and in all lymph nodes. Deer given the full dose of vaccine had a more pronounced visceral infection.

Examinations made 90 days after the vaccination showed that Br. abortus strain 19 persisted in 100% of deer, irrespective of the dose. Not all vaccinated animals had Brucella organisms in the viscera or lymph nodes. No Brucella organisms could be detected in deer slaughtered after eight months.

Pathogenic Br. abortus strain 010 obtained from deer was used for comparison and control of infection in vaccinated deer. This strain was first studied in the Brucellosis Laboratory of the Siberian Scientific Research Veterinary Institute (NIVI - Nauchno-Issledovatel'skii Veterinarnyi Institut).^{*} Of ten guinea pigs, two each were given 500, 100, 50, 5 and 2 micro-organisms (numbers determined by nephelometry).

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The numbers of microorganisms in prepared suspensions were checked by inoculation on Beef-Liver Peptone Agar. Thirty days after infection the guinea pigs were sacrificed. Cultures were obtained from all guinea pigs, irrespective of the strength of the initial vaccine dose. The minimum dose producing brucellosis in deer was determined next.

Deer (18 head) were divided into six groups (a stag, a doe and a grown calf in each group). The following numbers of *Br. abortus* strain 010 (as determined nephelometrically) were injected subcutaneously in the neck region of deer of each of the respective groups: 10^4 , 10^5 , 5×10^3 , 5×10^2 , 2×10^2 [the last group served as control].

On the 20th to 25th day after infection the deer showed the following symptoms: depression, partial loss of appetite and rumination, slight inflammation at the site of injection, considerable enlargement of intermaxillary, prescapular and inguinal lymph nodes, elevated body temperatures (to 40.6°), emaciation. Thirty days after injection, deer were slaughtered. Pathological examination revealed that all the lymph nodes were enlarged and hyperemic, with punctate and striated hemorrhages, and necrotic areas. The spleen was dense and roughened, dry and with grayish nodules under the spleen capsule. The uterine mucosa was inflamed, the bladder hyperemic in places.

Material from dissections were sown in agar and on nutrient broth (pH 6.8 - 7.0). These cultures were incubated for a month at 37° . Cultures of *Brucella* were obtained from all deer (with the single exception of one infected with 1×10^6 microorganisms), irrespective of the dosage.

To check the intensity of acquired immunity, healthy deer (36 head) were divided into three groups, all vaccinated with *Br. abortus* strain 19: in the first group (9 head) 6×10^{10} microorganisms each; in the second group (11 head), 15×10^9 ; in the third group (15 head: 10 adults and 5 calves $1\frac{1}{2}$ months old), 6×10^9 .

Ten months after *Br. abortus* strain 19 vaccination, deer were injected with 10^4 *Br. abortus* strain 010 microorganisms.

Simultaneously, seven healthy, unvaccinated deer were infected with an equal dose of the pathogen for comparison. Some of these last deer, when examined for agglutinin on the 10th, 20th and 30th day, showed only an insignificant increase in serum agglutinin titer. The animals were in depressed condition and showed loss of appetite; intermaxillary and prescapular lymph nodes were enlarged and body temperatures rose to 41° . Agglutination reaction on the 30th day was in the 1:50 - 1:800 titer range.

After 30 to 33 days vaccinated and unvaccinated animals were killed. In the vaccinated deer there were no visible changes in the viscera or lymph nodes (except for diapedesis and occasional reddening of the lymph nodes).

Cultures on Beef-Liver Peptone Agar and broth were prepared from lymph nodes, parenchymatic tissues, blood, bone marrow, uterine mucosa, fetal tissue and urine. Of the three vaccinated groups, animals from the first group proved resistant to infection. *Brucella* cultures were obtained from mediastinal and prescapular lymph nodes from only two individuals. Three deer of the second group developed partial immunity (in one animal *Brucella* cultures were obtained from the prescapular, in another from the mediastinal, and in a third from the intramaxillary, epiglottal and inguinal lymph nodes); in the third group, immunity was established in four deer. *Brucella* cultures were obtained in one animal from epiglottal

and scrotal lymph nodes and from the spleen, in a second and third animal from aural and axillary lymph nodes, and in a fourth animal from the spleen. Considerable *Brucella* cultures were obtained from viscera and lymph nodes of the unvaccinated deer.

The intensity of immunity in vaccinated animals was in effect practically the same in deer of all three groups.

In some deer immunized with one-quarter and one-tenth doses, a generalized form of brucellosis was observed.

Conclusions

1. Reindeer proved highly sensitive to the weakly pathogenic *Br. abortus* strain 19.

2. *Br. abortus* strain 19 vaccine is harmless to healthy deer and to deer with positive agglutination reaction. The immunobiological adjustment of the vaccinated animal is expressed by the appearance of agglutinins in blood serum 3 days after vaccination.

3. In 80% of vaccinated deer, immunity is obtained for eleven months (whole period of observation).

4. Pathogenic *Br. abortus* strain 010 obtained from deer in the Yakut ASSR (Davydov) is highly virulent on injection to deer and guinea pigs.

5. In view of violent post-vaccination reactions produced by 6×10^{10} *Br. abortus* strain 19, which produces an immunity equal to that induced by smaller dosages (15 or 16×10^9), research must be continued to determine the optimum vaccine dose.



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